論　文　の　内　容　の　要　旨

獣医学　専攻
平成 ２４ 年度博士課程 入学
氏　名　トンタｰブ　アティガン
指導教員名　中山　裕之

論文題目　　Pathological studies on canine histiocytic tumors
（犬の組織球性腫瘍に関する病理学的研究）

Histiocytes originate from $CD34^{+}$ bone marrow precursor stem cells through the control of a variety of bioactive features, such as granulocyte-macrophage colony stimulating factor (GM-CSF), macrophage colony stimulating factor (M-CSF), and tumor necrotic factor-α (TNF-α). Histiocytes are commonly found in the ubiquitous tissues and play important roles in the antigen presentation and phagocytosis in both innate and adaptive immune responses. In general, histiocytes can be divided into 2 major cell types; myeloid dendritic cells (DC) and macrophages based on a variety of cytokines released, cell-surface markerss and their primary functions. Histiocytic proliferative disorders (HPDs) in the dogs were first described in literatures in 1970s. Recently, the disorders are categorized into reactive histiocytosis, cutaneous histiocytoma and histiocytic sarcoma (HS) based on clinical behaviors and pathological characteristics.

This study focuses on canine HS, a malignant form of HPDs. It is a fatal and aggressive hematopoietic neoplasm with a worsen prognosis. This tumor occurs most frequently in middle-aged or older purebred dogs, particularly in Bernese mountain dogs, Retrievers and Rottweilers. However, the incidence of HS in young dogs is also reported in Perro de Presa Canario dogs and Great danes. Most of canine HS cases originate from DC, and cases deriving from macrophage, namely hemophagocytic HS are very rare. All the canine HS cases are also classified into localized and disseminated forms. The former is recognized as a solitary mass that mainly manifests in the skin and subcutis of the extremity with local invasion of sentinel lymph nodes, whereas the latter mainly appears as multiple lesions in the spleen, lung and bone marrow with the presence of wide dissemination. The lesions of HS are

preferentially observed in the skin, lymphoid tissues and other visceral organs, whilst the incidence of those in the central nervous system (CNS) is very low in both human and animals. The cellular origin and histogenesis of HS with CNS involvement is still unclear. Moreover, microscopic morphology of HS sometimes closely resembles other hematopoietic tumors (i.e. anaplastic plasmacytoma and anaplastic large cell lymphoma), malignant melanoma, and undifferentiated carcinoma. Thereby, pathological diagnosis of HS is challenging and immunohistochemical (IHC) confirmation is needed. The aims of this study are as follows; 1) to describe comparative pathological features of canine HS derived from neural and extraneural origins, 2) to unveil histological and IHC characteristics of primary intracranial canine HS, and 3) to elucidate biological, cellular morphological and immunophenotypic features of 2 newly established HS cell lines isolated from brain and synovial tumors, respectively.

In Chapter 1, fifty-four canine HS samples were categorized into HS with CNS involvement (HS-CNS; n = 23) and HS without CNS involvement (HS-NCNS; n = 31). The Pembroke Welsh Corgi and Flat coated retriever are high risk breeds for HS with CNS and non-CNS involvement, respectively, suggesting that the certain genetic factors may contribute to HS development in the dog. Microscopically, most of the HS cases contained numerous round- to pleomorphic-shaped cells. These cells had clear to eosinophilic cytoplasm with distinct borders. Moderate to marked atypia was noted. Bizarre mononuclear and multinucleated giant tumor cells interspersing within tumor lesions were commonly found. Immunohistochemically, all the 54 tumors were positive for HLA-DR, Iba-1 and CD204, 42 for CD163, 22 for lysozyme, 17 for S100 and 25 for CD208. None of cases were positive for myeloid/histiocyte antigen (MAC387). Considering the IHC expression pattern, HS cells from CNS tend to exhibit the macrophage phenotype whereas those from other organ systems show the dendritic cell differentiation. Interestingly, although histological and IHC features of HS between the 2 categories are not different, the proliferative parameter, mitotic index (MI), of HS-CNS group had a significantly higher average MI than those of HS-NCNS ($p<0.05$), supporting the aggressiveness of primary HS in the CNS.

In Chapter 2, clinicopathological, histological and immunohistochemical characteristics of intracranial HS in 23 dogs are described in details. Magnetic resonance imaging (MRI) and/or computed tomography (CT) for the brains revealed that the tumors were mainly located in the cerebrum, particularly in the frontal lobe. Seizure was a predominant clinical sign in most of the cases. Tumor cells were morphologically classified into the round/polygonal- and the spindle-shaped cell types. Interestingly,

hemophagocytic activity of the round/polygonal cell type was significantly higher than that of the spindle cell type ($p<0.05$), suggesting that the biological behaviors of the round/polygonal-shaped cell type are more aggressive than the other. However, there was no significant difference in other clinicopathological parameters and mitotic index between the 2 types. Immunohistochemically, tumor cells were strongly positive for HLA-DR, Iba-1 and CD204 in all the 23, for iNOS in 20, for CD163 in 17, for lysozyme and CD208 (DC-LAMP) in 9, and for S100 in 5 cases. These observations suggest that canine primary intracranial HS tend to exhibit both dendritic cell and/or macrophage phenotypes with histiocytic differentiation.

In Chapter 3, two novel canine HS cell lines (PWC-HS01 and FCR-HS02) isolated from brain and synovial tumors, respectively, were established. Phagocytic ability was observed in both established cell lines. The average of modal chromosome numbers of PWC-HS01 and FCR-HS02 cell lines were 74.5 and 45.4, respectively, indicating hypoploidy. Cellular morphology of both cell lines were identical. However, the results of immunocytochemical and reverse transcription-polymerase chain reaction showed that PWC-HS01 expressed both DC and macrophage markers, whereas the majority of FCR-HS02 expressed DC markers. Furthermore, both cell lines were intensely positive for hematopoietic stem and progenitor cell markers. In accordance with the expression of hematopoietic stem cell markers, cultured HS cells reverted into their progenitors or the stem cell (dedifferentiation), but the dedifferentiated HS cells can be redifferentiated into the other cell phenotype through *in vivo* reproliferation with a devoid of hematopoietic stem cell markers. For *in vivo* assays, these two newly established HS cells were also tumorigenic and metastatic when injected into immunodeficient mice, suggesting that these cell lines can be used as new tumor models for canine HS.

In conclusion, HS has a highly aggressive behavior and a poor outcome due to the rapid systemic dissemination at the time of diagnosis. Thereby, early diagnosis of this tumor is highly required. Although the incidence of primary canine intracranial HS is very low, HS cells originated from CNS commonly share both DC and macrophage differentiation, unlike HS from other organ systems. These present observations indicate that biological behaviors of primary HS in the CNS is indeed aggressive and the prognosis is quite poor.